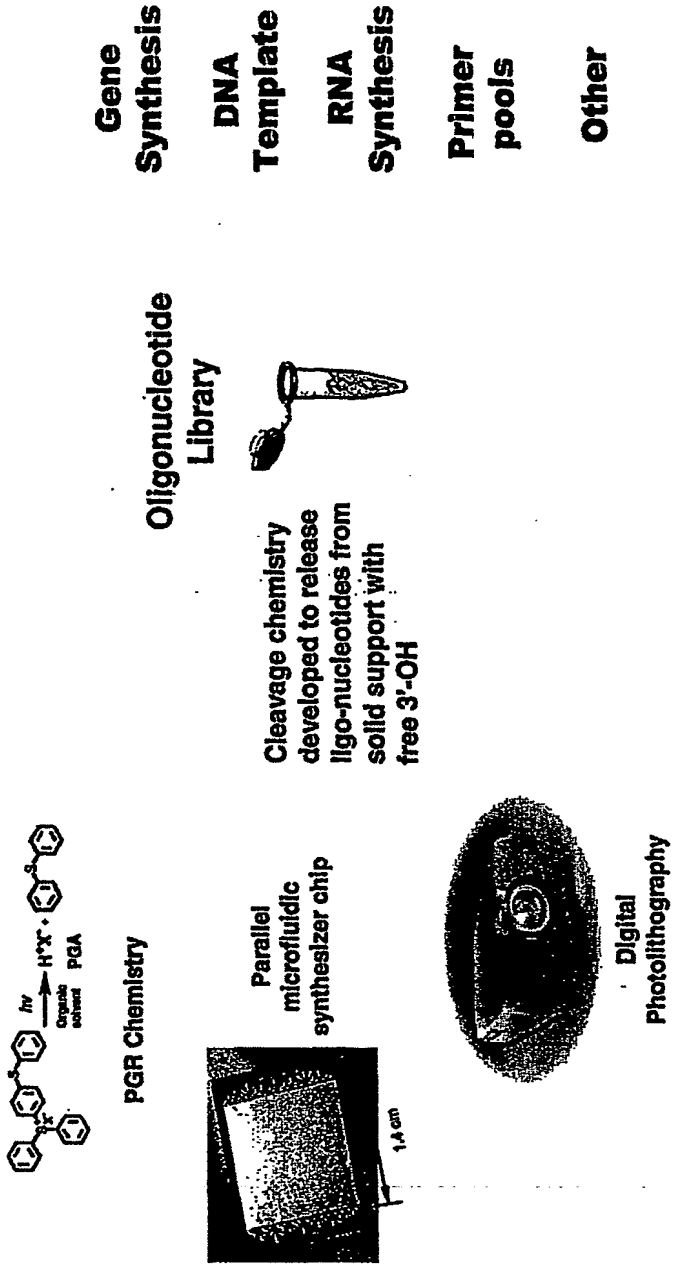


Figure 1

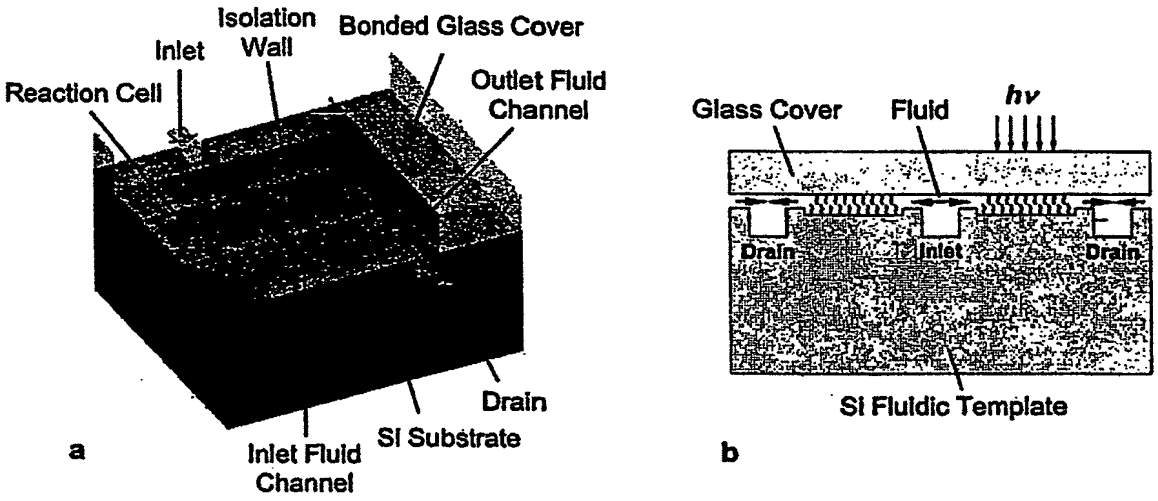

Isolation Wall
Bonded Glass Cover
Inlet
Outlet Fluid Channel
Reaction Cell
Drain
Si Substrate
Inlet Fluid Channel
a
Glass Cover
Fluid
hv
Drain
Inlet
Drain
Si Fluidic Template
b

Figure 2

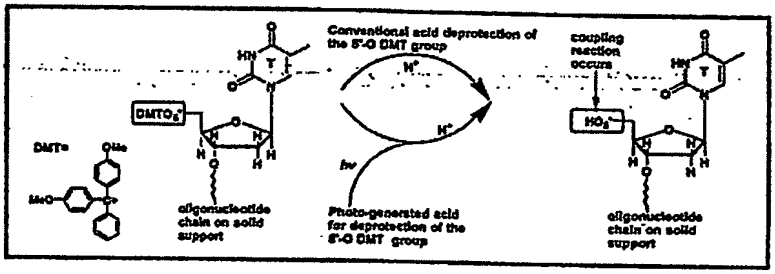

Conventional acid deprotection of the 5'-O DMT group
coupling reaction occurs
DMTO
DMT+
oligonucleotide chain on solid support
hv
Photo-generated acid for deprotection of the 5'-O DMT group
HO
oligonucleotide chain on solid support

Figure 3

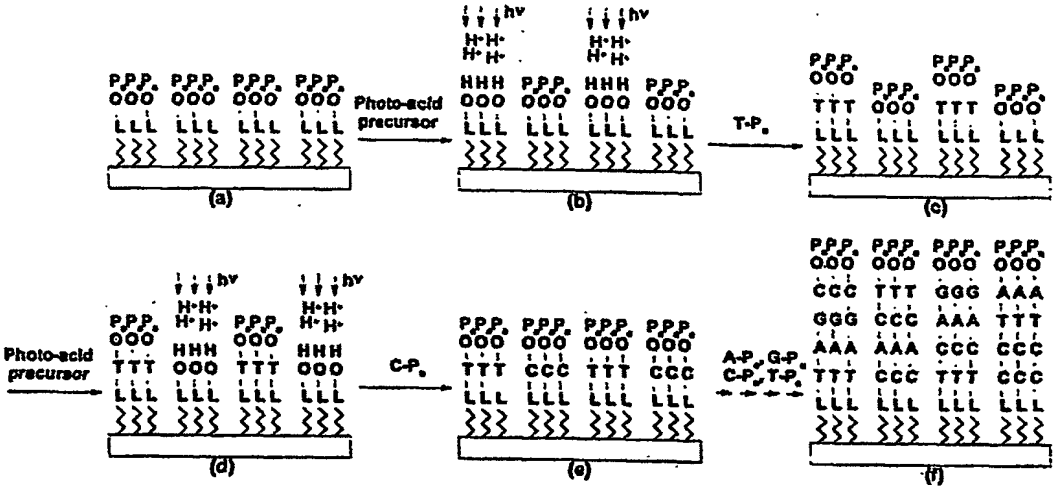


Figure 4

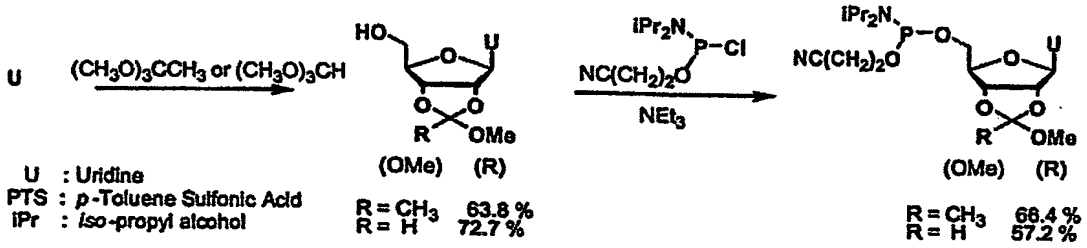

U
(CH3O)3CCH3 or (CH3O)3CH
HO
U
O
O
R
OMe
(OMe) (R)
iPr2N
P–Cl
NC(CH2)2O
NEt3
iPr2N
P–O
NC(CH2)2O
U
O
O
R
OMe
(OMe) (R)
U : Uridine
PTS : p-Toluene Sulfonic Acid
iPr : Iso-propyl alcohol
R = CH3 63.8 %
R = H 72.7 %
R = CH3 66.4 %
R = H 57.2 %

Figure 5

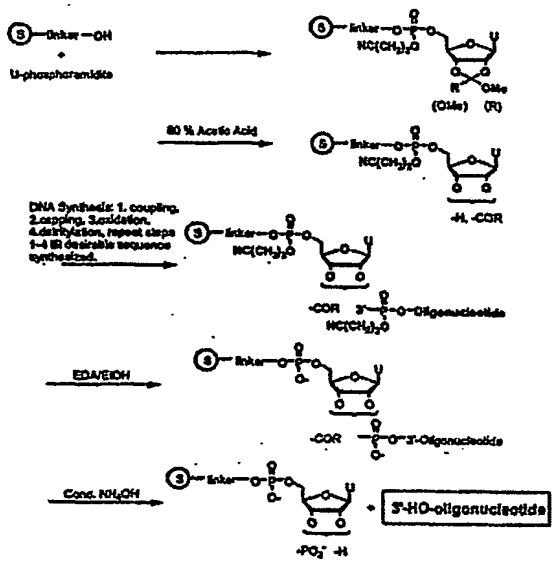

Linker—OH
U-phosphoramidite
80 % Acetic Acid
DNA Synthesis: 1. coupling, 2.capping, 3.oxidation, 4.detritylation, repeat steps 1-4 till desirable sequence synthesized.
EDA/EtOH
Conc. NH4OH
3'-HO-oligonucleotide

Figure 6

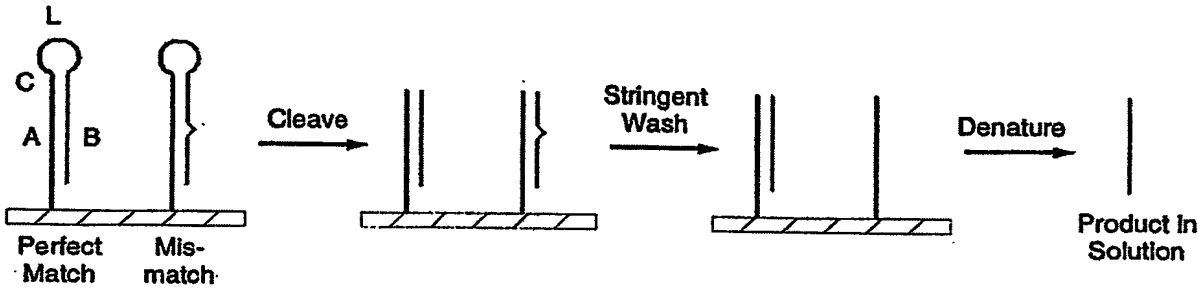

L
C
A
B
Perfect Match
Mis-match
Cleave
Stringent Wash
Denature
Product in Solution

Figure 7

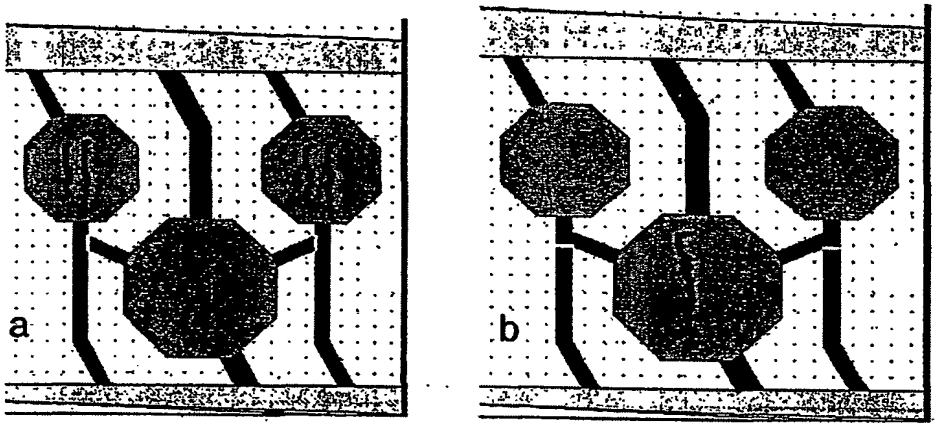


Figure 8

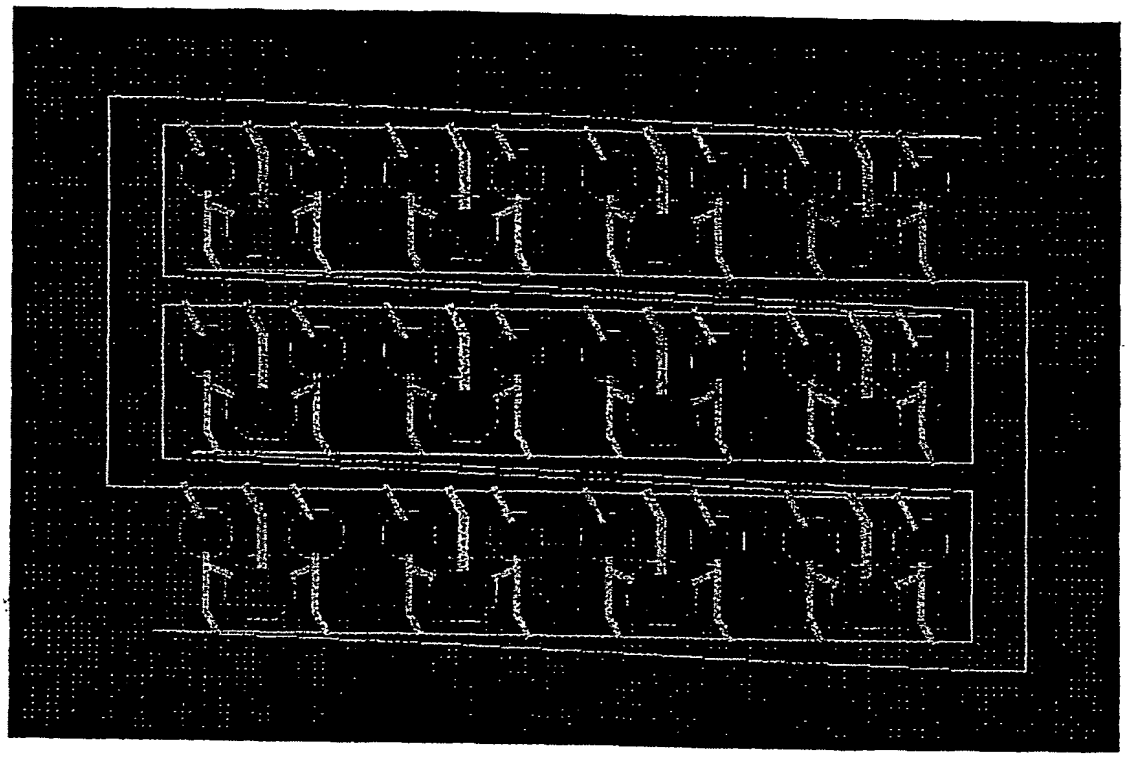


Figure 9

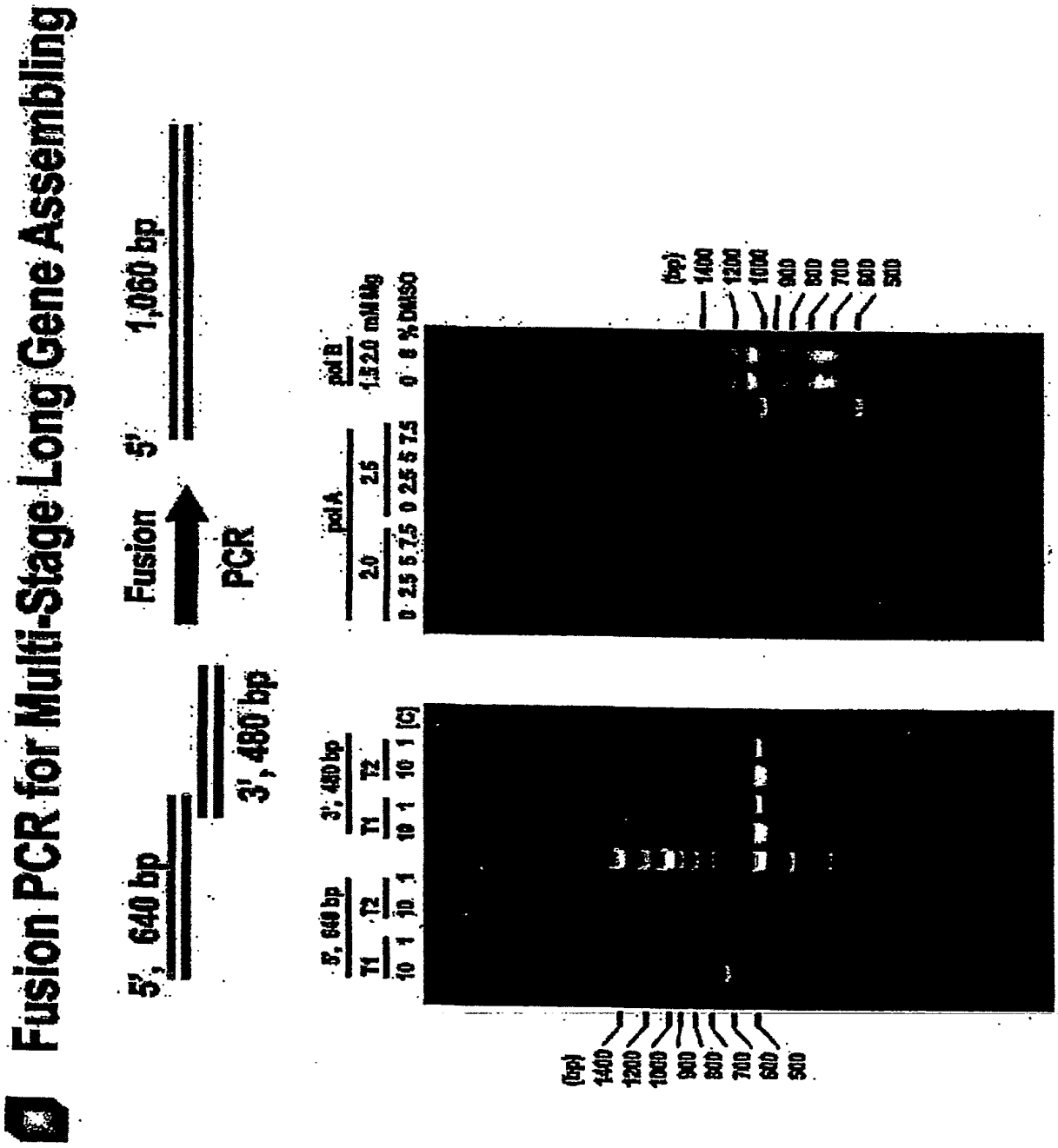

Fusion PCR for Multi-Stage Long Gene Assembling
5', 640 bp
3', 480 bp
Fusion PCR
5' 1,060 bp
pol A
pol B
(bp)
1400
1200
1000
900
800
700
600
500

Figure 10

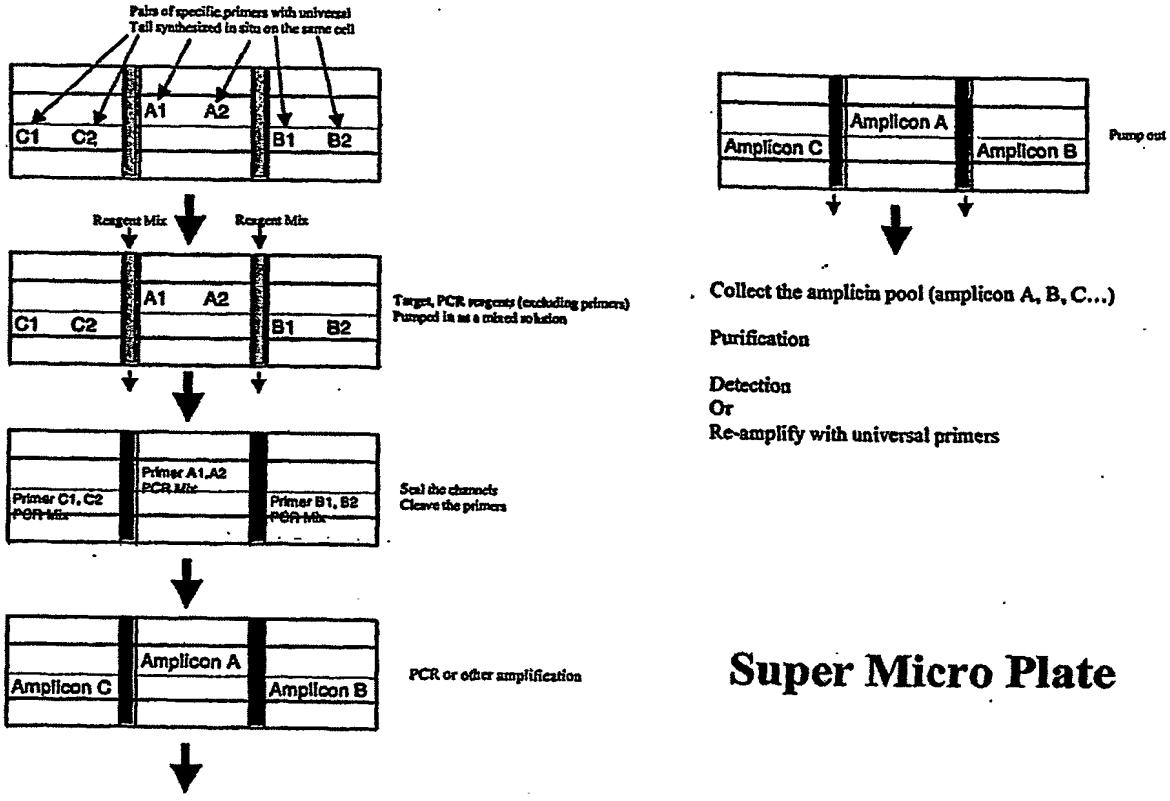

Pairs of specific primers with universal Tail synthesized in situ on the same cell
C1
C2
A1
A2
B1
B2
Reagent Mix
Reagent Mix
Target, PCR reagents (excluding primers) Pumped in as a mixed solution
Primer C1, C2 PCR Mix
Primer A1,A2 PCR Mix
Primer B1, B2 PCR Mix
Seal the channels Cleave the primers
Amplicon C
Amplicon A
Amplicon B
PCR or other amplification
Amplicon C
Amplicon A
Amplicon B
Pump out
Collect the amplicin pool (amplicon A, B, C...)
Purification
Detection
Or
Re-amplify with universal primers
Super Micro Plate

Figure 11

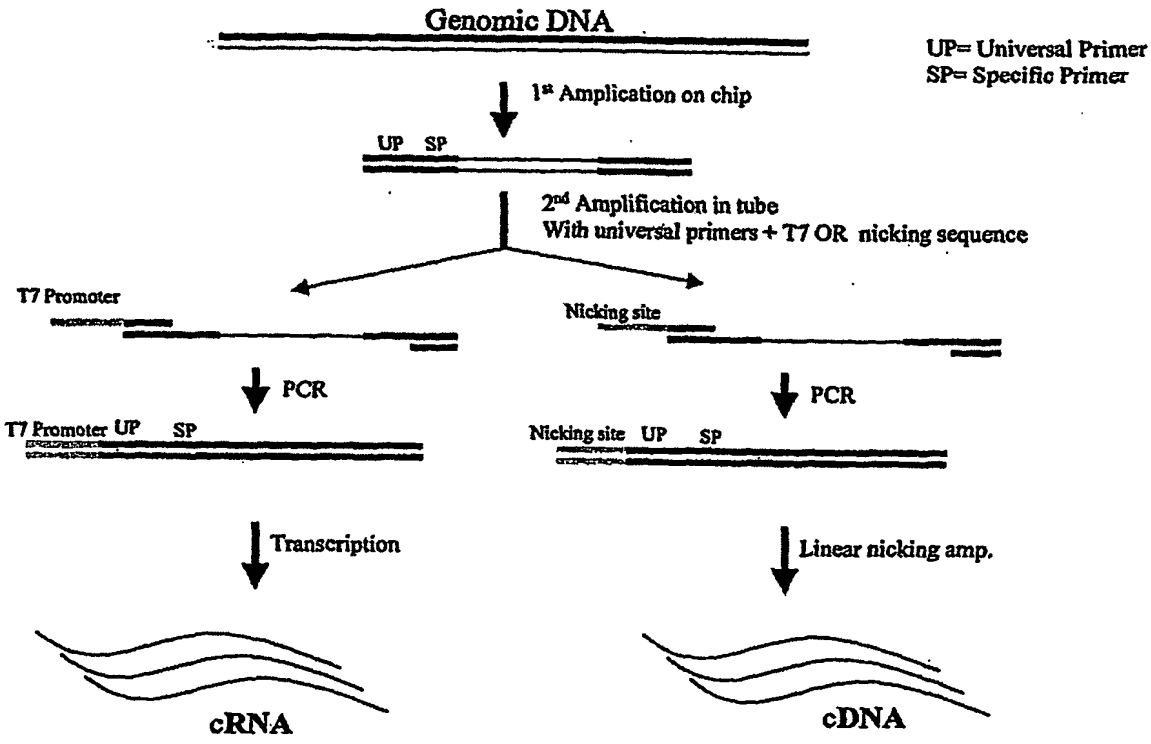

Genomic DNA
UP= Universal Primer
SP= Specific Primer
1st Amplication on chip
UP SP
2nd Amplification in tube
With universal primers + T7 OR nicking sequence
T7 Promoter
Nicking site
PCR
PCR
T7 Promoter UP SP
Nicking site UP SP
Transcription
Linear nicking amp.
cRNA
cDNA

Figure 12

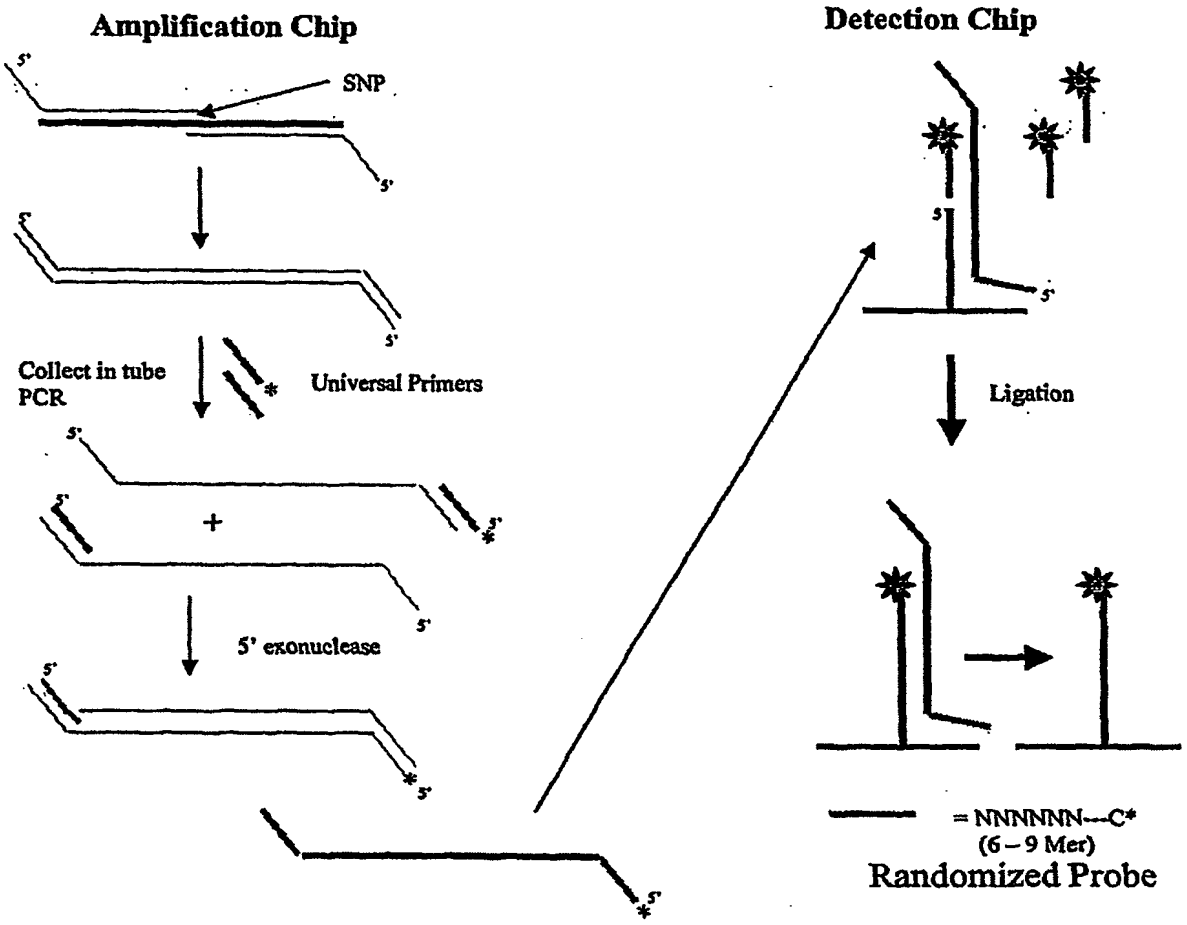

Amplification Chip
SNP
Collect in tube
PCR
Universal Primers
5' exonuclease
Detection Chip
Ligation
= NNNNNN---C*
(6 – 9 Mer)
Randomized Probe

Figure 13

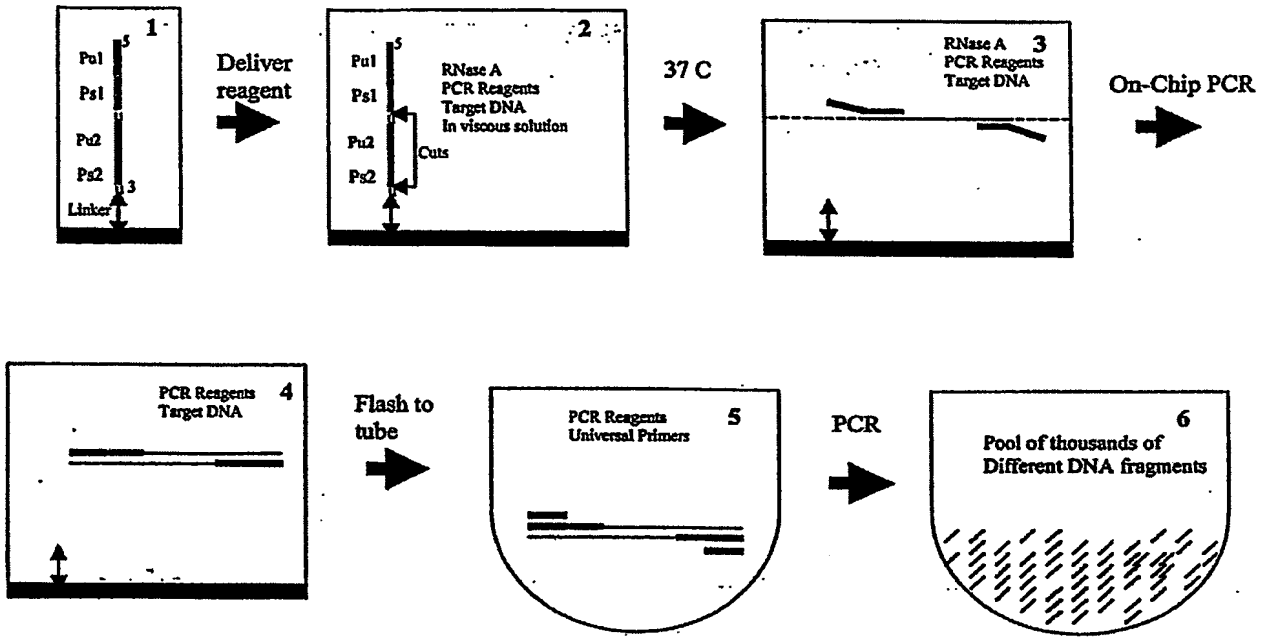
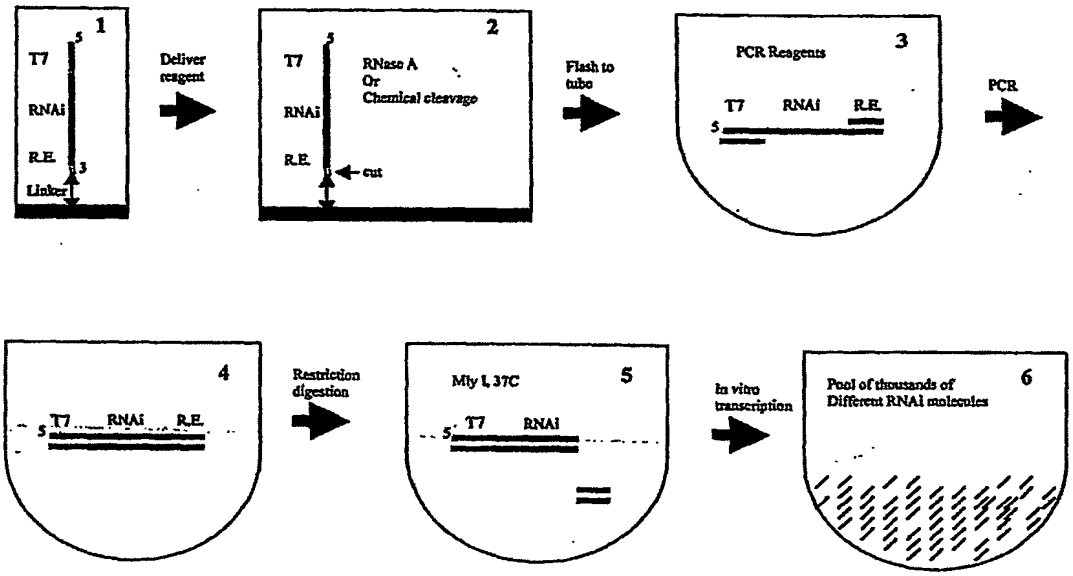

1
Pu1
Ps1
Pu2
Ps2
Linker
Deliver reagent
2
RNase A
PCR Reagents
Target DNA
In viscous solution
Cuts
37 C
3
RNase A
PCR Reagents
Target DNA
On-Chip PCR
4
PCR Reagents
Target DNA
Flash to tube
5
PCR Reagents
Universal Primers
PCR
6
Pool of thousands of
Different DNA fragments

Figure 14



1. In situ synthesized oligos (about 60mer). Each oligo contains a rU, one T7 promoter, specific RNAi sequence and a Mly I restriction site. Mly I is a blunt cutting enzyme (GAGTCNNNNN^).
2. Oligos are cleaved by RNase A or chemicals.
3. Flash the oligos into tube. PCR amplify the targets with universal primers, one contains the T7 sequence and the other contains the restriction sequence.
4. Double stranded PCR products with thousands of specific RNAi sequences and common T7 and restriction site.
5. Digest the PCR products with Mly I enzyme to remove the tail.
6. In vitro transcription to produce unlimited supply of RNAi pools (can be re-amplified and re-transcribed) ready for use.

Figure 15

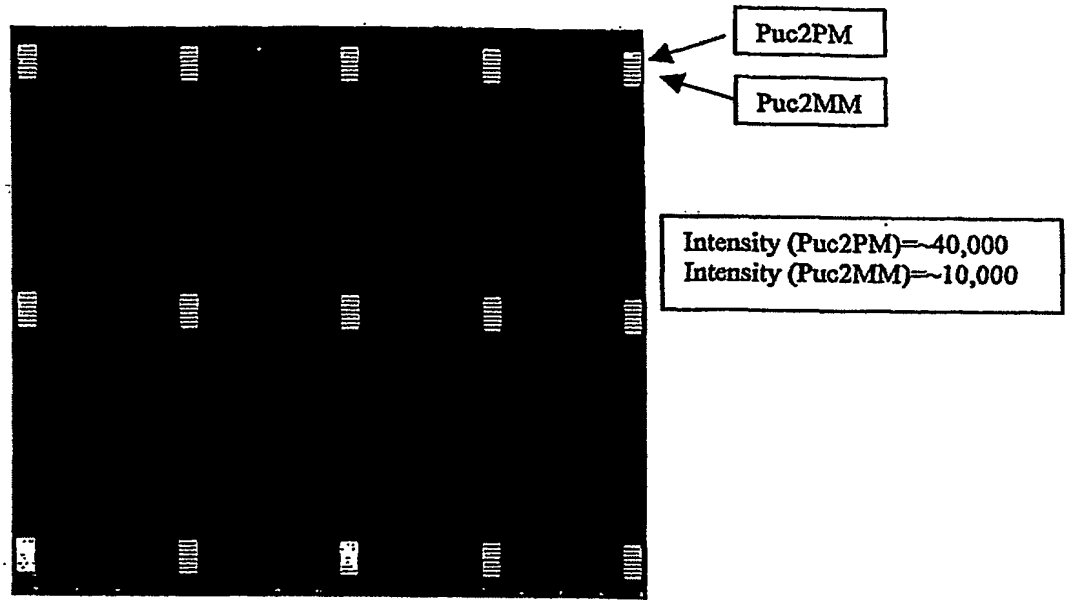

Puc2PM
Puc2MM
Intensity (Puc2PM)=~40,000
Intensity (Puc2MM)=~10,000

Figure 16

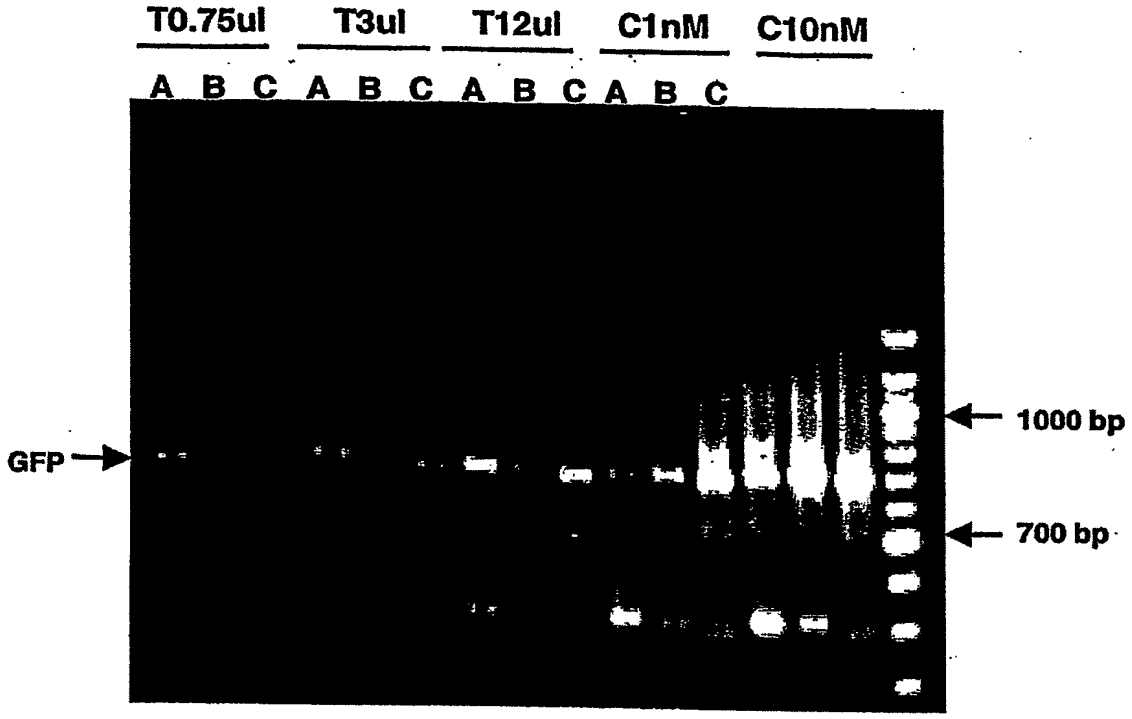

T0.75ul
T3ul
T12ul
C1nM
C10nM
A B C A B C A B C A B C
GFP
1000 bp
700 bp

Figure 17

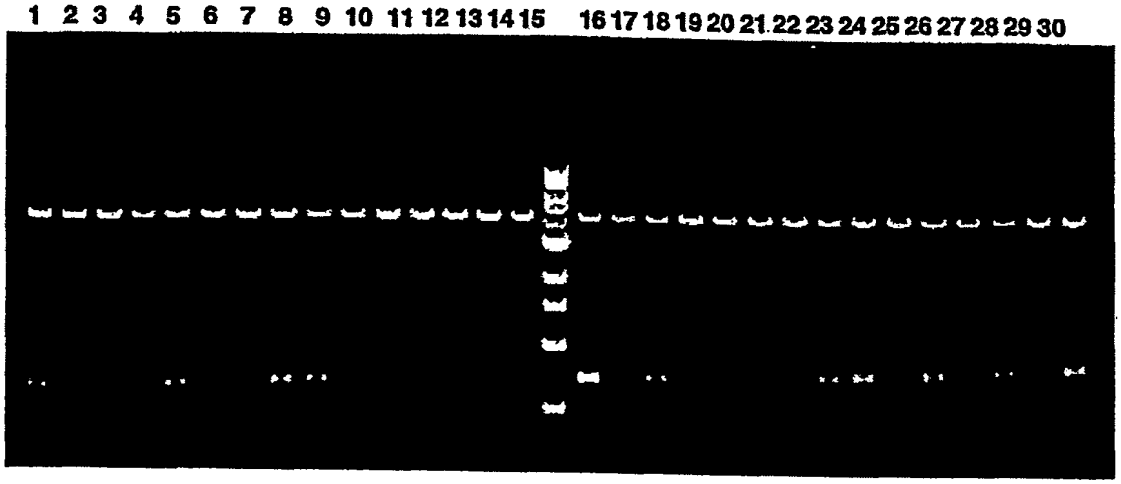
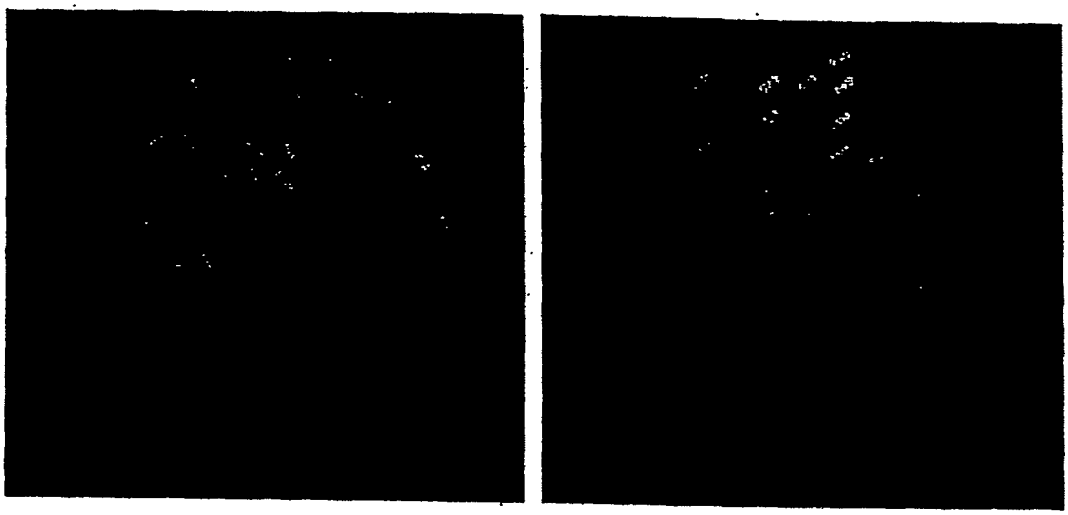

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Figure 18



Dish 1 Dish 2

Figure 19

Figure 20

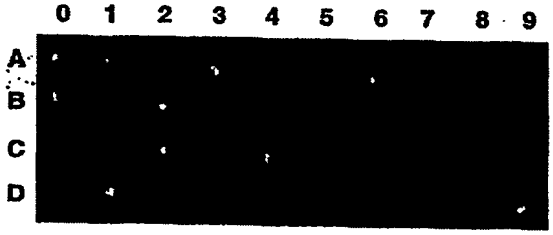


Figure 21

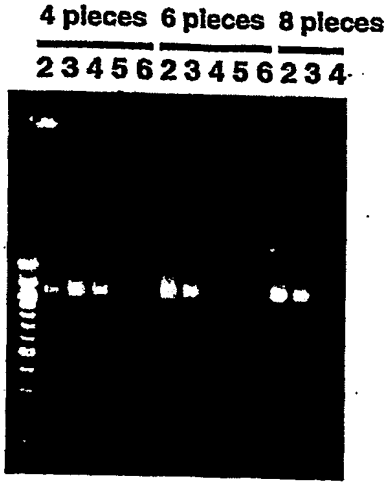


Figure 22

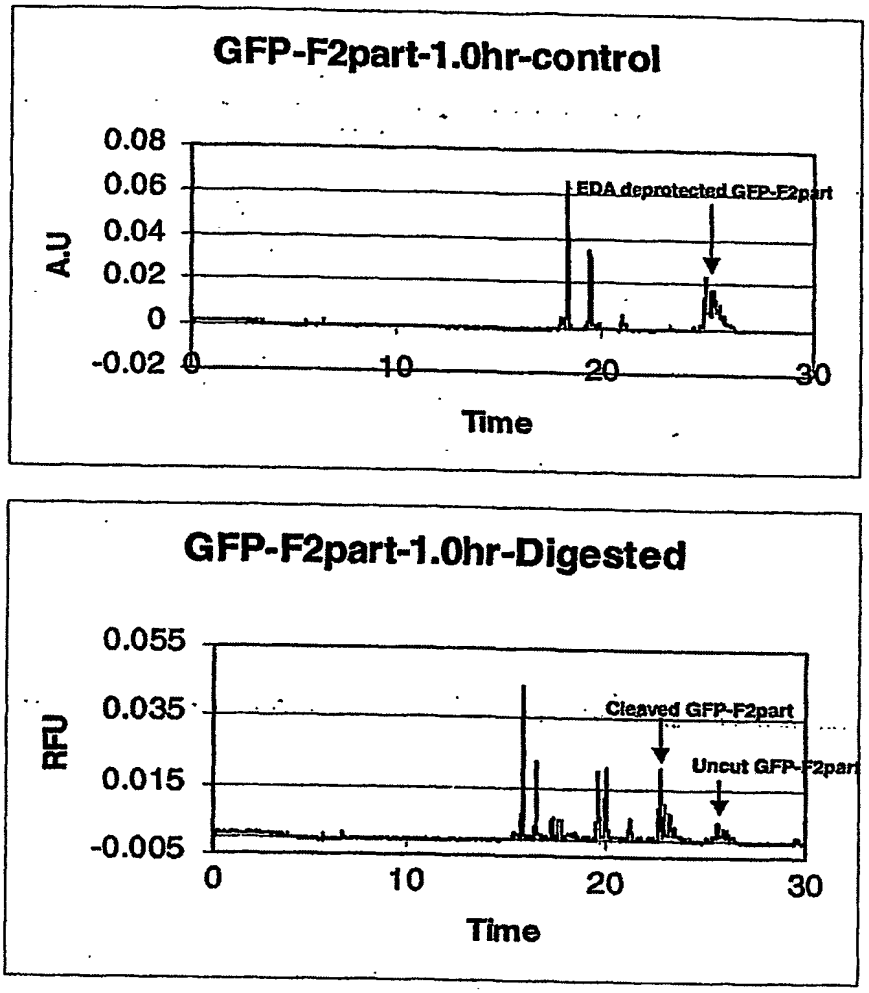

GFP-F2part-1.0hr-control
A.U
0.08
0.06
0.04
0.02
0
-0.02
0
10
20
30
Time
EDA deprotected GFP-F2part
GFP-F2part-1.0hr-Digested
RFU
0.055
0.035
0.015
-0.005
0
10
20
30
Time
Cleaved GFP-F2part
Uncut GFP-F2part

Figure 23

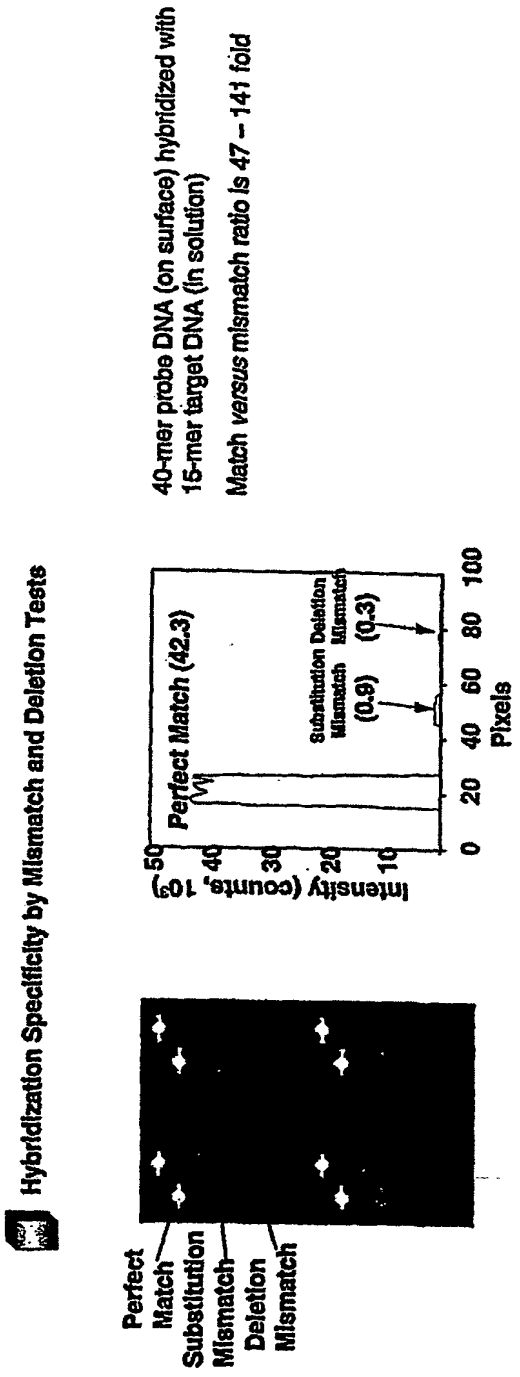

Hybridization Specificity by Mismatch and Deletion Tests
40-mer probe DNA (on surface) hybridized with 15-mer target DNA (in solution)
Match versus mismatch ratio is 47 – 141 fold
Perfect Match (42.3)
Substitution Mismatch (0.9)
Deletion Mismatch (0.3)
Intensity (counts, 10³)
Pixels
Perfect Match
Substitution Mismatch
Deletion Mismatch

Figure 24

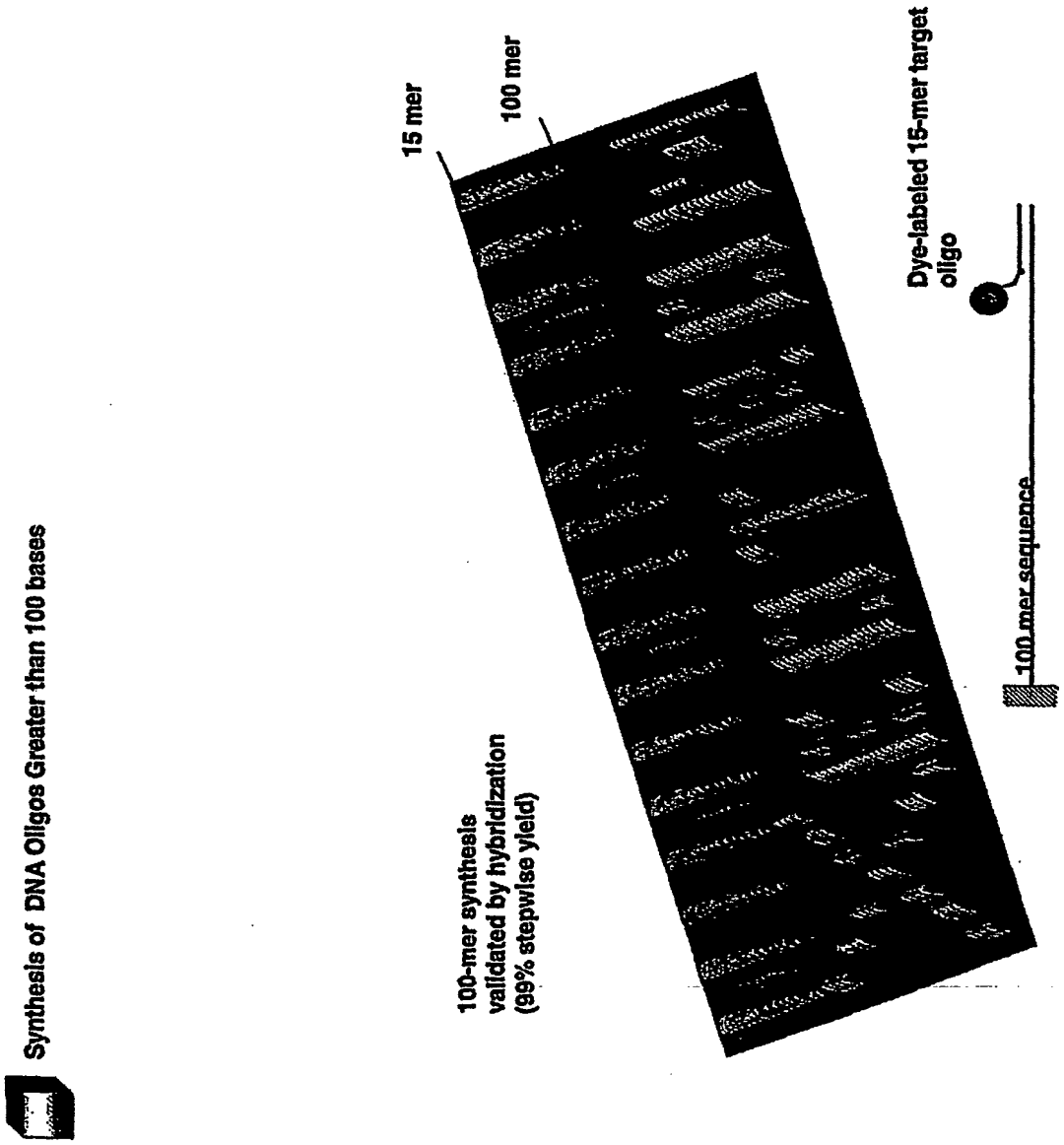

Synthesis of DNA Oligos Greater than 100 bases
100-mer synthesis validated by hybridization (99% stepwise yield)
15 mer
100 mer
Dye-labeled 15-mer target oligo
100 mer sequence

Figure 25

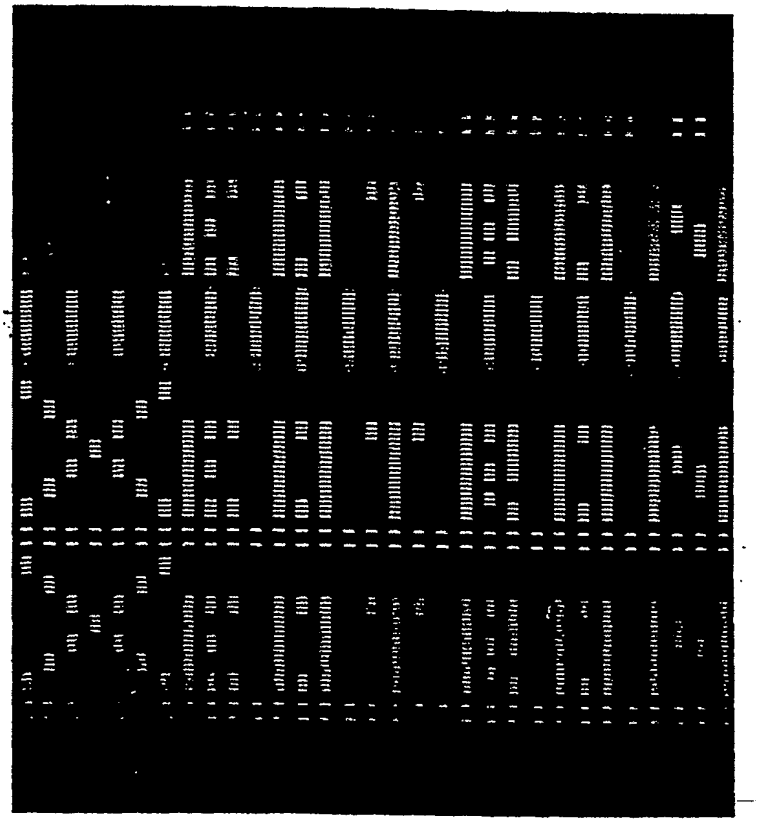


Figure 26

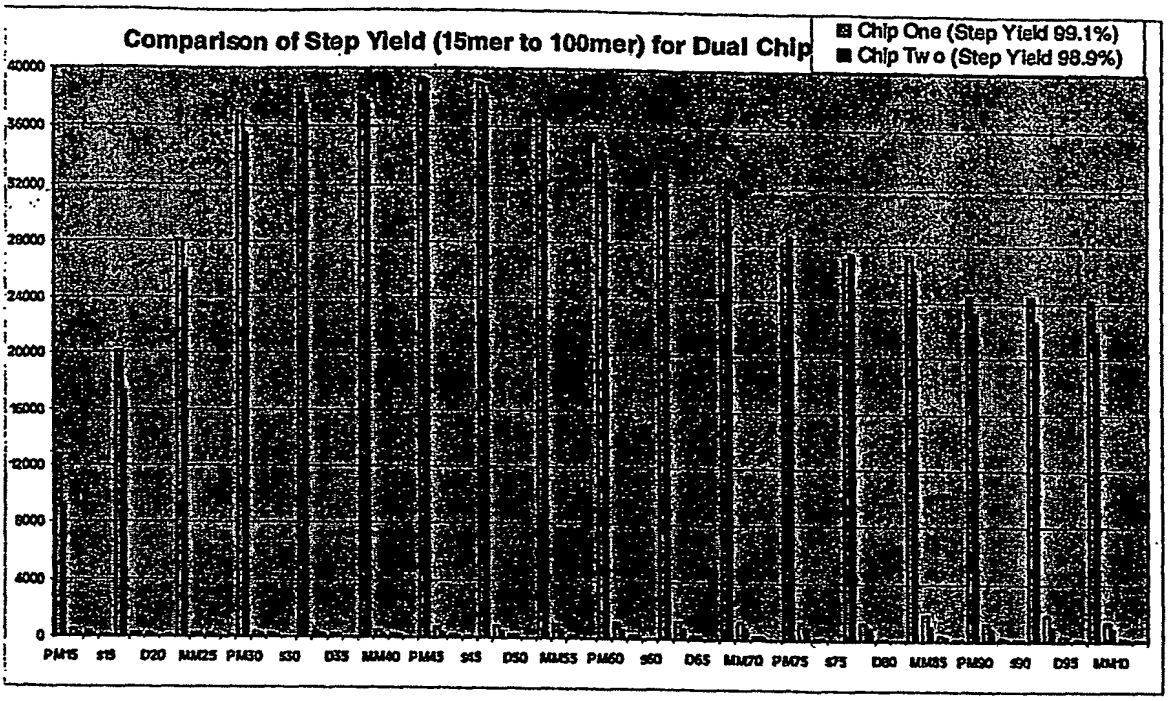

Comparison of Step Yield (15mer to 100mer) for Dual Chip
Chip One (Step Yield 99.1%)
Chip Two (Step Yield 98.9%)
40000
36000
32000
28000
24000
20000
16000
12000
8000
4000
0
PM15 S15 D20 MM25 PM30 S30 D35 MM40 PM45 S45 D50 MM55 PM60 S60 D65 MM70 PM75 S75 D80 MM85 PM90 S90 D95 MM10

Figure 27

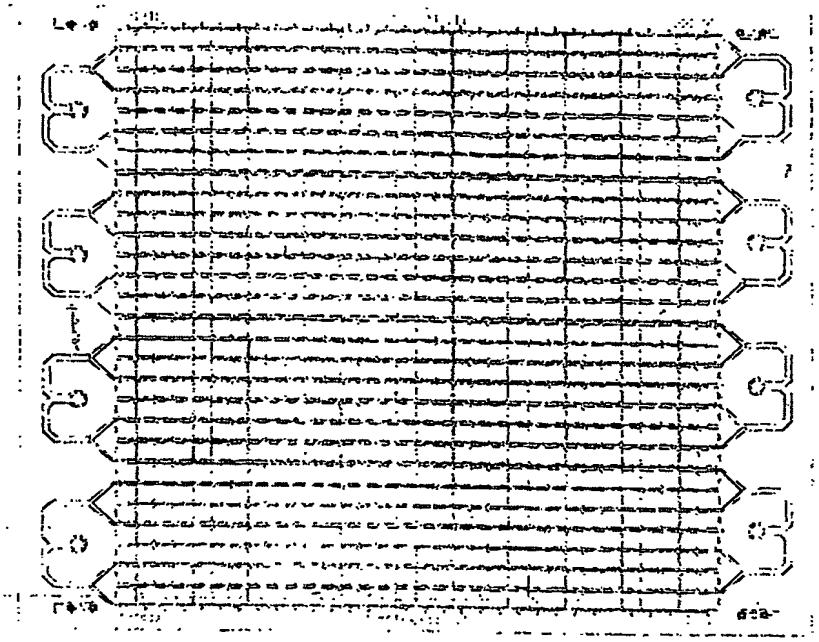


Figure 28

Electrophoresis gel analysis of PCR sequences (60 mers) and the sequences after SAP1 enzyme digestion (41 and 19 mers)

Figure 29

Rec'd PCT/PTO 28 APR 2005

PCT/US2003/034207

10/533208

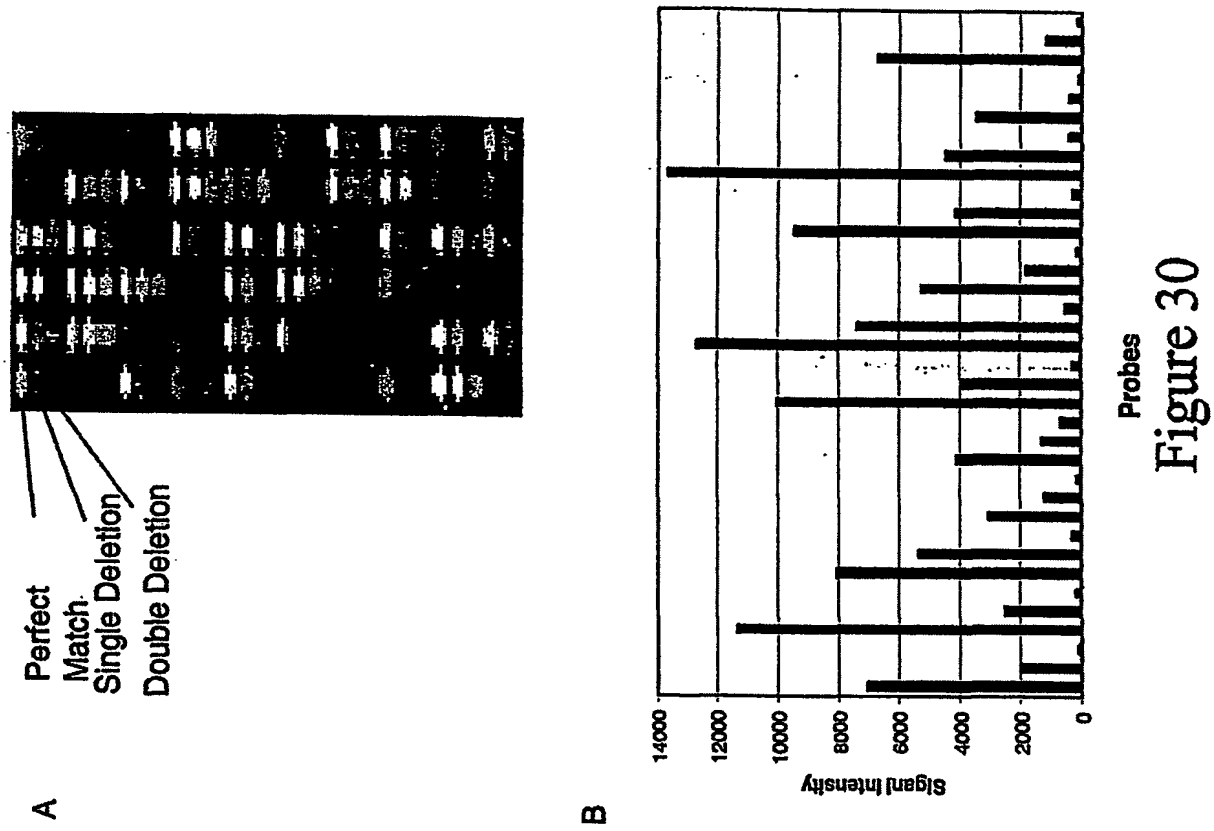


Figure 30